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Study Of Blinking Statistics In Silver Coated Cobalt Ferrite Single Nanoparticle Using Two-Photon Fluorescence Microscopy

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STUDY OF BLINKING STATISTICS IN SILVER COATED COBALT FERRITE SINGLE NANOPARTICLE USING TWO-PHOTON FLUORESCENCE MICROSCOPY

RAJEN KUMAR GOUTAM

Master’s Program in Physics

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Stephen L. Crites, Jr., Ph.D.
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Rajen Kumar Goutam

2019
STUDY OF BLINKING STATISTICS IN SILVER COATED COBALT FERRITE SINGLE
NANOPARTICLE USING TWO-PHOTON FLUORESCENCE MICROSCOPY

by

RAJEN KUMAR GOUTAM

THESIS

Presented to the Faculty of the Graduate School of
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of the Requirements
for the Degree of

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Department of Physics
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Abstract

Two-photon fluorescence microscopy is a powerful tool to study the molecular and cellular interactions. This technology is a non-invasive approach with the advantage of three-dimensional imaging up to submicron resolution. Two-photon excitation process is the result of simultaneous absorption of two photons that has special features of reduced photodamaged and elongated penetration depth on samples. In this work, blinking statistics of silver coated Cobalt ferrite (CoFe₂O₄) single nanocrystal is studied using two-photon fluorescence microscopy. By defining the intensity threshold, the observed fluorescence is divided into two distinct stages: OFF and ON states. Both of these states followed inverse power law. This power law is different from the well-known three-level quantum jump for blinking.
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Chapter 1: Two-Photon Absorption

1.1 THEORY OF TWO-PHOTON ABSORPTION

The first prediction of simultaneous absorption was done in 1931 by Göppert Mayer at her thesis. It is fundamental radiation-matter interaction in which excitation of atoms or molecules from lower quantum state\( |1 > \) to an excited state\( |2 > \) of same parity take place. [1] In this phenomenon atomic system is assumed to be quantized and radiation is treated classically. The probability of this quantum transition is proportional to the cross-section of atoms or molecules and to the square of excitation intensity of the incoming field. We use time dependent perturbation theory [2] to calculate the probability of such transition.

We consider Hamiltonian (\( \hat{H} \)) that consist of two parts;

\[
\hat{H} = \hat{H}_0 + \hat{V}(t)
\]  

(1.1)

Where \( \hat{H}_0 \) does not depend on time explicitly called unperturbed Hamiltonian and \( \hat{V}(t) \) is the interaction energy with the applied optical field. The interaction energy be defined as

\[
\hat{V}(t) = -\hat{\mu}\hat{E}(t)
\]  

(1.2)

With, \( \hat{\mu} = -e\hat{r} \)  

(1.3)

Once Hamiltonian (\( \hat{H} \)) is known for \( t > 0 \), we need to solve the time evolution wave function \( \psi \) in the presence of time dependent potential \( V(t) \). Now the Hamiltonian wave equation

\[
\hat{H}\psi = i\hbar \frac{\partial \psi}{\partial t}
\]  

(1.4)

To solve equation (1.4) we begin by writing

\[
\psi_1 = u_1e^{-\frac{E_1t}{\hbar}} \text{ and } \psi_2 = u_2e^{-\frac{E_2t}{\hbar}}
\]
That satisfy the stationary state solution

\[ \hat{H}_0 U_i = E_i U_i \quad (1.5) \]

\[ \text{i=1,2} \]

Under the influence of em, the wave function can be written as

\[ \psi = a_1(t)\psi_1 + a_2(t)\psi_2 \quad (1.6) \]

where \( a_1 \) and \( a_2 \) are time dependent probability amplitude complex number satisfying the completeness axiom

\[ |a_1|^2 + |a_2|^2 = 1 \quad (1.7) \]

Defining the absorption rate

\[ V_{km} = -\frac{d|a_k(t)|^2}{dt} = \frac{d|a_m(t)|^2}{dt} \quad (1.8) \]

So, we need to find \( |a_2(t)|^2 \) which is done by writing the solution of (1.6) as

\[ \psi = \sum_{k} a_k \psi_k = \sum_{k} a_k u_k e^{-i\frac{E_k t}{\hbar}} \quad (1.9) \]

where \( k \) denotes the general state of the atom and \( m \) gives number of these states. Applying (1.9) on (1.6)

\[ \sum_{k}(\hat{H}_0 + \hat{V}(t))a_k u_k e^{-i\frac{E_k t}{\hbar}} = \sum_{k}\{i\hbar \left( \frac{d a_k}{dt} \right) u_k e^{-i\frac{E_k t}{\hbar}} + a_k u_k E_k e^{-i\frac{E_k t}{\hbar}} \} \quad (1.10) \]

With the stationary solution of (1.5) we get,

\[ \sum_{k}\{i\hbar \left( \frac{d a_k}{dt} \right) u_k e^{-i\frac{E_k t}{\hbar}} = \sum_{k} a_k \hat{V}(t) u_k e^{-i\frac{E_k t}{\hbar}} \quad (1.11) \]

Multiplying both side by \( u_n^* \), arbitrary eigen function and integrating overall space, we get
\[
\sum \{ \text{i} \hbar \left( \frac{da_k}{dt} \right) u_k e^{-\frac{i(E_k t)}{\hbar}} \} \int u_k u^*_m dV = \sum a_k e^{-\frac{i(E_k t)}{\hbar}} \int u^*_m \hat{V}(t) u_k dV \tag{1.12}
\]

Using orthonormality condition
\[
\int u_k u^*_n dV = \delta_{kn}
\]
equation (1.12) gives

\[
\left( \frac{da_m}{dt} \right) = \frac{1}{\text{i} \hbar} \sum_1^m \hat{V}_{mk} a_k e^{-\frac{i(E_k - E_m) t}{\hbar}} \tag{1.13}
\]

Where
\[
\hat{V}_{mk} = \hat{V}_{mk}(t) = \int u^*_m \hat{V}(t) u_k dV \tag{1.14}
\]

Equation (1.14) consists a set of m differential equations for m variables \(a_k(t)\). Now using the perturbation theory, i.e. \(a_m(t) = a^0_m(t) + \lambda a^1_m(t) + \lambda^2 a^2_m(t) + \cdots\)

These equations can be solved if the initial conditions are known. In our case we take two level systems. The wavefunction \(\psi\) using equation (1.6) and (1.12) reduces to

\[
\left( \frac{da^1_m}{dt} \right) = \frac{1}{\text{i} \hbar} \left\{ \sum_k \hat{V}_{mk} a^0_k(t) e^{-\frac{i(E_k - E_m) t}{\hbar}} \right\} \tag{1.15i}
\]

\[
\left( \frac{da^2_m(t)}{dt} \right) = \frac{1}{\text{i} \hbar} \left\{ \sum_k a^1_k(t) \hat{V}_{mk} e^{-\frac{i(E_k - E_m) t}{\hbar}} \right\} \tag{1.15ii}
\]

\[
\left( \frac{da^N_m(t)}{dt} \right) = \frac{1}{\text{i} \hbar} \left\{ \sum_k a^{N-1}_k(t) \hat{V}_{mk} e^{-\frac{i(E_k - E_m) t}{\hbar}} \right\} , N = 1,2,3, \ldots \ldots \tag{1.15iii}
\]
Which must be solve by using the initial conditions $a_g^0(t) = 1, a_k^0(t) = 0$. $k \neq g$ for N=1 gives the linear absorption. With equation (1.2) and (1.3)

$$\hat{V}_{mg} = -\mu_{mg}(Ee^{-i\omega t} + E^* e^{-i\omega t})$$

Where

$$E = Ee^{-i\omega t} + E^* e^{-i\omega t}$$

is the applied monochromatic field, So (1.15.i) becomes,

$$\left(\frac{da_m^1(t)}{dt}\right) = -\frac{1}{i\hbar}\mu_{mg}(Ee^{-i\omega t} + E^* e^{i\omega t})e^{i\omega_{mg}t}$$

We consider

$$\frac{(E_k - E_m)}{\hbar} = \omega_{mk} = \omega_{mg}$$

Integrating we get

$$a_m^1(t) = \frac{\mu_{mg}}{\hbar(\omega_{mg} - \omega)}E[e^{i(\omega_{mg} - \omega)t} - 1] + \frac{\mu_{mg}}{\hbar(\omega_{mg} + \omega)}E^*[e^{i(\omega_{mg} + \omega)t} - 1]$$

(1.16)

If $\omega \approx \omega_{mg}$ only the first term in equation (1.16) dominates to give absorption of photon.

$$a_m^1(t) \approx \frac{\mu_{mg}}{\hbar(\omega_{mg} - \omega)}E[e^{i(\omega_{mg} - \omega)t} - 1]$$

which is resonant for one photon absorption. The process of two-photon absorption is obtained when N=2,

$$\frac{da_n^2(t)}{dt} = \frac{1}{i\hbar}\left\{ \sum_m a_m^1(t)\hat{V}_{nm} e^{[i\omega_{nm}]t} \right\}$$

With

$$\hat{V}_{nm} \approx -\mu_{nm}E^{-i\omega t}$$

(1.17)

on solving and using (1.16) gives
\[
\frac{da_n^2(t)}{dt} \approx \frac{1}{\hbar} \sum_m \frac{\mu_{mn} \mu_{mg} E^2}{\hbar (\omega_{mg} - \omega)} e^{i(\omega_{ng} - 2\omega)t}
\]

Integrating,

\[
a_n^2(t) = \sum_m \frac{\mu_{mn} \mu_{mg} E^2}{\hbar^2 (\omega_{mg} - \omega)} \left[ e^{i(\omega_{ng} - 2\omega)t} - 1 \right]
\]

which is two photon absorption process represented by the following figure 1.1.

![Fig:1.1 Two-photon absorption labeling several levels](image)

Now to get the probability, we take the square of probability amplitude \(a_n^2(t)\) that an atom at state \(n\) at time \(t\) given by

\[
p_n^2(t) = |a_n^2(t)|^2 = \left| \sum_m \frac{\mu_{mn} \mu_{mg} E^2}{\hbar^2 (\omega_{mg} - \omega)} \right|^2 \left| e^{i(\omega_{mg} - 2\omega)t} - 1 \right|^2
\]

(1.18)

The numerator of second terms can be expand and simplify to obtain

\[
\left| e^{i(\omega_{mg} - 2\omega)t} - 1 \right|^2 = 4\sin^2 \left( \frac{\omega_{ng} - 2\omega}{2} t \right)
\]

(1.19)

So, we have

\[
p_n^2(t) = \left| \sum_m \frac{\mu_{mn} \mu_{mg} E^2}{\hbar^2 (\omega_{mg} - \omega)} \right|^2 \left| 4\sin^2 \left( \frac{\omega_{ng} - 2\omega}{2} t \right) \right|
\]

(1.20)
\[ p_n^2(t) = \left| \sum m_{\mu n \mu m g} E_2 \right|^2 f(t) \]  
(1.21)

Where

\[ f(t) = 4\sin^2 \left( \frac{(\omega_{ng} - 2\omega)t}{2} \right) \]

The function, \( f(t) = 4\sin^2 \left( \frac{(\omega_{ng} - 2\omega)t}{2} \right) \) is plotted against \( (\omega_{ng} - 2\omega) \). One sees that the peak value of the function becomes greater as it’s width narrower and \( t \) increases as shown below in fig 1.2.

![Graph of \( f(t) \) against \( (\omega_{ng} - 2\omega) \)]

**Fig:1.2** Plot of \( f(t) \) against \( \omega_{ng} - 2\omega \) (Dirac delta function)

When, \( \omega_{ng} = 2\omega \) the peak value of \( t^2 \) is reached and the width of central peak is of the order of \( \frac{4\pi}{t} \).

The area can be written as \( A = \frac{1}{2} \cdot 4\pi \cdot t^2 = 2\pi t \) for larger \( t \), the function become extremely high and can be expressed as Dirac delta function i.e.
\[
\lim_{t \to \infty} f(t) = 2\pi t(\omega_{ng} - 2\omega)
\]
so, the probability becomes
\[
p_n^2(t) = \left| \sum_m \frac{\mu_{mn} \mu_{mg} E^2}{\hbar^2 (\omega_{mg} - \omega)} \right|^2 2\pi t \delta(\omega_{ng} - 2\omega)
\]

(1.22)

We can express the state \( n \) in terms of density of final state \( \rho_f \omega_{ng} \), defined between transition frequencies \( \omega_{ng} \) and \( \omega_{ng} + d\omega_{ng} \). It is necessary to average over all possible values of transition frequency

\[
p_n^2(t) = \left| \sum_m \frac{\mu_{mn} \mu_{mg} E^2}{\hbar^2 (\omega_{mg} - \omega)} \right|^2 2\pi t \int_0^\infty \rho_f (\omega_{ng}) \delta(\omega_{ng} - 2\omega) d\omega_{ng}
\]

Or

\[
p_n^2(t) = \left| \sum_m \frac{\mu_{mn} \mu_{mg} E^2}{\hbar^2 (\omega_{mg} - \omega)} \right|^2 2\pi \rho_f (\omega_{ng} = 2\omega)
\]

which can be express in terms of two-photon cross section

\[
\sigma_{ng}^2(\omega) = \frac{1}{(2\eta \varepsilon_0 c)^2} \left| \sum_m \frac{\mu_{mn} \mu_{mg} E^2}{\hbar^2 (\omega_{mg} - \omega)} \right|^2 2\pi \rho_f (\omega_{ng} = 2\omega)
\]

and with intensity of incident beam,

\[
I = 2\eta \varepsilon_0 c |E|^2
\]

\[
p_n^2(t) = \sigma_{ng}^2(\omega) I^2 t
\]

(1.23)

From which probability of two-photon absorption depends on square of excitation intensity which is nonlinear and has vital role in optical sectioning. From equation (1.23) the cross section of local maxima should be double the wavelength used for single photon excitation, which is applicable for symmetric molecules only, not for complex structures. A typical magnitude of cross section is order of \( 10^{-10} \text{cm}^4\text{s} \text{photon}^{-2} \) as defined by Göppert Mayer and equation (1.23). So, photon of high flux densities from the source is necessary to recompense this small cross section. A tightly focused
beam from high power can be used to accomplished it, but it may result to heating effect. The only way to avoid the situation is to use short-pulsed laser and to combine spatial and temporal focusing of the beam. This may result to reduce power delivered and hence the damage caused by incident beam on the sample. [3] [4]

1.2 TWO-PHOTON FLUORESCENCE INTENSITY

Two photon fluorescence intensity $I_f(t)$ is given by

$$I_f(t) \propto Q \cdot \sigma_{ng}^2 \cdot I^2(t) \tag{1.24}$$

Where $I(t)$ is excitation intensity, $\sigma_{ng}^2$ is the molecular cross-section, Q is the quantum yield. Again, the excitation intensity $I(t)$ is given by

$$I(t) = \frac{\lambda_{exc} P(t)}{hcA} \tag{1.25}$$

With $P(t)$ is the instantaneous power delivered at an illumination area A, defined as

$$A = \pi \left[ \frac{\lambda_{exc}}{2NA} \right]^2$$

$\lambda_{exc}$ is excitation radiation wavelength, NA the numerical aperture of objective lens, h is the Planck’s constant and c be the velocity of light. Substituting (1.25) in (1.24) we get

$$I_f(t) = D \cdot \pi \cdot \sigma_{ng}^2 \cdot Q \cdot P(t)^2 \left[ \frac{(NA)^2}{hc\lambda_{exc}} \right]^2$$

where D is proportionality constant. The time average two-photon fluorescence $I_f(t)$ can be expressed in terms of peak laser power when pulsed laser is used as

$$< I_{f, pulsed} > \approx \sigma_{ng}^2 \cdot Q \cdot \left[ \frac{(NA)^2}{hc\lambda_{exc}} \right]^2 \int_0^{\tau_p} P(t)^2_{peak} dt$$

relating peak power laser and average laser power as

$$P_{peak}(t) = \frac{P_{ave}}{\tau_p f_p}$$
in which $\tau_p$ is the pulse width and $f_p$ is the pulse repetition rate. Thus, in terms of average laser power two photon fluorescence intensity can be expressed as

$$< I_{f,\text{pulsed}} > \equiv \sigma_{ng}^2 Q \frac{(NA)^2}{h \lambda_{exc}} \frac{1}{T} \int_0^{\tau_p} P_{ave}^2 \frac{1}{\tau_p f_p^2} dt \equiv \sigma_{ng}^2 Q \frac{(NA)^2}{h \lambda_{exc}} \frac{P_{ave}^2}{\tau_p f_p}$$

Fig: 1.3 Energy transition occurring in two-photon emission

1.3 LIGHT SOURCE

We have femtosecond titanium-sapphire (Ti:Sapphire) laser source. The laser provides pulses with duration on the order of 100fs with rate of 80MHz. The average power can reach above 2.5W while it delivers peak powers of over 300kW. It can be tuned to wide range from 690nm to 1040nm of wavelength that allows selective excitation of a wide variety of fluorophores. The most
commonly used fluorophores for single photon excitation have wavelength ranging from 350nm to 690nm. The excitation wavelength for two-photon absorption can be approximated as twice the wavelength used for single photon. We are in the near-infrared region of the spectrum. Similarly, the absorption coefficients in the range of wavelengths provided by our laser source in most biological specimens are minimized in this range. In other words, there is low absorption and scattering in this wavelength range accounts for greater penetration depth in biological samples.

1.4 TWO-PHOTON LASER SCANNING MICROSCOPE

![Diagram of Two-Photon Laser Scanning Fluorescence Microscope]

Fig: 1.4 Schematic of the Two-Photon Laser Scanning Fluorescence Microscope developed in the Bio-photonics Laboratory of the Physics Department at UTEP.

1.5 WAVEPLATE AND POLARIZER

The light is linearly polarized in the horizontal direction when it releases from the laser source. Immediate after the source there is a half waveplate (HWP) attached to a rotary mounting
which allows us to shift the polarization direction by rotating the fast and slow axis of the waveplate. A typical half waveplate (HWP) introduces a phase shift of $\pi$ between the polarization components. For linearly polarized light this translates into a rotation of $2\theta$, where $\theta$ is defined as the angle formed by the polarization vector and the fast axis of the waveplate.

After the half waveplate there is a linear polarizer that splits the beam into two parts with different linear polarization. This type of polarizer is more suitable for use with our femtosecond laser since it does not need to absorb the high intensity light; instead it allows a particular polarization direction to go through parallel to the direction of the beam while all other polarization directions are deflected perpendicular to the beam path, we use this combination of waveplate and polarizer to effectively control the average power of our laser beam. Usually, the polarizer is kept in a fixed position while the wave plate is rotated to obtain the desired average power. This can be easily explained from Malus’ law which describes the intensity of a beam when a perfect polarizer is placed in its path and is given by

$$I = I_0 (\cos \alpha)^2$$

Where $I_0$ and $I$ are the intensities of the laser beam before and after the polarizer and $\alpha$ is the angle formed between the polarization vector coming out of the waveplate and the axis of the polarizer. In an ideal case the full intensity is transmitted when $\alpha = 0^\circ$ while the light beam is blocked if $\alpha = 90^\circ$

1.6 SCANNING PLATFORM

The scanning mechanism and its timing circuit board are two important part of our two-photon system. It provides the rapid scanning capability that is very crucial to image dynamic cellular processes and also it is suitable for live animal studies. It can be imaged a full frame of $350\mu\text{m}$ by $350\mu\text{m}$ at a rate of 30 frames per second currently. With the addition of an electronic
circuit, it can be tuned to the frame rate to 60 and 120 frames per second, that allow further faster processes. The field of view decreases by one half of its full frame dimension in the case of 60 frames per second and by one fourth for 120 frames per second.

The scanning platform is built with a galvanometer mounted mirror and a spinning polygonal mirror that produce a raster scan pattern. The galvanometer mounted mirror scans in the vertical direction whereas the spinning polygonal mirror scans the horizontal direction. The polygonal mirror has a 36 facets disk equally distributed along the edge; it has constant rotational speed of 480 revolutions per second. Each facet corresponds to a line in the image parallel to the x axis. It can be calculated easily the line scanning rate for the polygonal mirror, that is fixed to 17280 Hz. Following Table 1.1 provides the amount of lines per image calculated for each frame rate.

<table>
<thead>
<tr>
<th>Frame rate (Hz)</th>
<th>Lines per image</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>576</td>
</tr>
<tr>
<td>60</td>
<td>288</td>
</tr>
<tr>
<td>120</td>
<td>144</td>
</tr>
</tbody>
</table>

The number of lines per images are integer multiple of the number of facets (36) guarantees the fact that the same facet scans the same line on every successive frame preventing vertical scrolling effects.

But the galvanometer-mounted mirror can be scanned in the vertical dimension at rate of 30 Hz, 60 Hz or 120 Hz, therefore setting the scanning rate of the microscope. to synchronize the galvanometer and polygonal mirrors a bicell photodiode is used to count the number of facets (lines) and generate an electronic signal which is used by the circuitry to drive the galvanometer at a particular frequency. The photodiode is able to generate the synchronization signal by means
of a 650nm laser diode that shines on the polygonal scanner in such a way that its reflection scans across the bicell photodiode.

1.7 DICROIC MIRRORS AND FILTERS

Different wavelengths laser beams are separated using Dichroic mirrors. They have the property of transmitting and reflecting a certain range of wavelength. In our microscope system as shown in fig 1.4 we have three dichroic mirrors labeled as a, b and c. In our set up the dichroic mirrors are positioned in such a manner that the angle formed by the incident light with the mirror is close to 45 degrees. Dichroic a reflects all the wavelengths below 660nm while transmitting wavelengths above it. This allows that the excitation light reaches the objective lens while the fluorescence emitted by the sample is reflected in a perpendicular direction to the initial beam path. The next two dichroic, b and c, separate the fluorescent signal, redirecting specific ranges of wavelengths to each detector (PMT). For instant dichroic b transmits wavelengths above 495nm, allowing the blue range of wavelengths to reach the PMT designated for the blue channel. The transmitted fluorescence continues a straight path until it reaches the dichroic c, where it is separated one more time; wavelengths below 580nm, containing the green portion of the spectrum, are reflected towards the green channel detector while the remaining fluorescent signal is transmitted towards the red channel detector. There are a couple of band pass filters in front of each detector represented in figure 1.4 and labeled as d, e and f which help to narrow down wavelength and selectively detect the fluorescent from the sample.

1.8 OBJECTIVE LENS

In our laboratory we have numerous objective lenses. Among which the two Olympus objective lenses differing mainly in the numerical aperture (NA) and the working distance. The
NA is a unitless number that characterizes the range of angles over which the objective lens can accept or emit light. It is defined as

\[ NA = n \sin \beta \]

Where \( \beta \) is the half angle of the maximum cone of light that can enter or exit the lens and \( n \) is the refractive index of the objective immersion liquid. The working distance is defined as the distance from the front lens element of the objective to the closest surface of the coverslip when the specimen is in sharp focus. As a general rule, the objective working distance decreases as the magnification and NA both increases.

Our two objective lenses have a magnification factor of 60X and a NA of 1 and 1.2. They are both water immersive objective lenses. The characteristics of the objective lens play an important role in determining the resolution of an imaging system. As it was explained before, optical sectioning is an intrinsic property of a two-photon system, giving it the capability of 3D imaging. The lateral resolution of our microscope can be approximated using Abbe’s equation

\[ R = \frac{0.6 \lambda}{NA} \approx \frac{\lambda}{2NA} \]

Where \( R \) is the minimum distance between distinguishable objects in an image and \( \lambda \) is the wavelength of the light used for illuminating the sample. This formula has its origin in the diffraction of light and the finite aperture of the optical elements; it represents a limit for the resolution of an optical system.

1.9 PHOTOMULTIPLIER TUBE (PMT)

The photomultiplier tubes are one of the main components of our system. They have a photosensitive surface to captures incident photons and generates electronic charges which are sensed and amplified. The current proportional to the number of photons striking the photosensitive surface is the output of a PMT.
The values for quantum efficiency (QE) may range between 20% and 40%. The quantum efficiency (QE) of these devices is the percentage of photons detected, is a function of the illumination wavelength and also depend on the chemical composition of the surface. PMTs respond within a few nanoseconds to changes in the input photon flux that making very reasonable to record and detect fast events. Although the dynamic range of these devices is also considerably wide, but the electrical output current accurately reflects the incident photon flux. The performance of electronic imaging sensors is signal to noise (SN) ratio. There is low dark current, which arises in electronic device in the absence of light, so the SN ratio is very high in PMTs. Each PMT is coupled with a Hamamatsu C7950 socket which converts the small-current high-impedance output of the PMT into a low-impedance voltage output with a conversion factor of \(0.3 \, \text{V/\mu A}\). Moreover, each C7950 is connected to a +/-15 and a variable power supply (0 V to +3.6 V) for high voltage adjustments. The latter is used to manually control the gain of the amplification circuit inside each C7950. PMTs allows effectively collecting of fluorescence because of larger area of photocathode. Even the fluorescence comes from a point in the sample at a time but each PMT is able to handle a maximum count rate of about 1 MHz in our current set up.

### 1.10 3D STAGE

As the scanning platform is the combination of the polygonal mirror and mounted galvanometer mirror produces a two-dimensional image at a given \(z\)-position. For creating a three-dimensional reconstruction of the sample, images have to be obtained at different points in the axial direction. This is achieved by our 3D motorized stage (Shutter Instrument, model MP-285). It can travel one inch on all three axes and provides a low resolution of 0.2 \(\mu\text{m/step}\) and a high resolution of 0.04 \(\mu\text{m/step}\). Mostly, the stack of image is taken at separation of 1 \(\mu\text{m/step}\) between
planes in our lab. The manufacturer for this stage has specified maximum speed of 2.9 μm/step which contributes to a small acquisition time.

1.11 TWO-PHOTON MICROSCOPY OVER ONE-PHOTON

When a fluorophore is illuminated by pulsed laser in two-photon, the intensity per pulse it will undergoes excitation is given by equation 1.26. The fluorescence signal intensity in two-photon microscopy is proportional to two-photon absorption cross section. Thus two-photon excited fluorescence can be spatially confined by focusing the excitation light.

Fig: 1.5 One photon vs two photon fluorescence imaging

Two-photon allows us to gain information in the axial direction as well, being three dimensional. Another important features of two-photon which is superior over one photon is the energy use for excitation as we use each of the photons provides only half of the required energy, which means that photons with far less energy than used in one photon, that can penetrate at deep tissue imaging. The wavelength is thus two times bigger than the wavelength of the fluorescence and the wavelength of light used in two-photon lies in Infra-red (IR) or near infra-red (NIR) which helps to reduce the photodamage on the samples.
Chapter 2: Magnetic Nanoparticles

Magnetic Nanoparticles (MNPs) are one of the most researched topics in last decade. MNPs have unique physical and chemical properties which not only differ with the bulk materials but also differ distinctly among themselves with the synthesizing process, particles size, distributions, shapes and compositions [5]. The reason behind much attention is their attractive properties which could be potential use in Nano-material based catalyst [6], Magnetic resonance imaging (MRI), biomedicine and tissue specific targeting, magnetic particle imaging [7], data storage, optoelectronics [8], Nano-fluids [9] magnetic separation, drug delivery, hyperthermia [10] and other various applications [7]. Fluorescent semiconductor nanocrystals, small crystalline substance also known as quantum dots are several nanometers in size. They contain about few hundreds of atoms Two important properties that scientists are interest on them are quantum confinement due to the size and light emission due to radiative recombination. With that two properties this nanocrystal is being used in labelling of biological molecules, sensitive imaging of cell, neuronal brain network, quantum dot displays (QDD) and potential use in laser operating over wide range from visible to near infrared (NIR).

2.1 PROPERTIES OF MNPS

MNPs have both mass and electric charge. The particles include holes, electrons, protons, and positive and negative ions. The two important features that dominate the properties of MNPs are: size effects and surface effects in which the former is due to quantum confinement of electrons whereas the latter is typically symmetry breaking of crystal structure at boundary. When electric charged particle spins, it creates magnetic dipole called magnetons. In ferromagnetic substances the magneton refers to group where the magnetic domain is associated with the volume (multi domain), whereas the single domain is associated with superparamagnetic. [11]
Domain walls separate regions of uniform magnetization within multi domain MNPs. Domain wall formation is governed by the balance between magneto-static energy $\Delta E_{MS}$ , that increases with increment of volume and domain wall energy $E_{dw}$, that increases with increasing interfacial area between domains. When the size of particle reaches below a critical diameter it become super paramagnetic with zero coercivity caused by thermal effect (Neel and Brownian motion). For spherical particles critical diameter is reached when energy $\Delta E_{MS} = E_{dw}$ [12]. This interesting feature of superparamagnetic state is that these particles become magnetic in the presence of external magnetic fields and revert to non-magnetic state on the removal of external field [13]. Large percentage of atoms are in the surface of nanoparticles on decreasing the particle size implying surface and interface effects are more important. The surface spin makes a large contribution in magnetization on such large surface atom ratio. Change in the band structure, lattice constant and or atom co-ordination might occur if the local symmetry is broken which leads to surface anisotropy and core-surface anisotropy on such particles [12].
2.2 SIZE DEPENDENT OPTICAL PROPERTIES

In bulk solids the optical properties do not alter typically with size which means when a sapphire is cut into smaller pieces its color remains same. But at nanoscale the color of material depends on its size. When the size of particle having red fluorescence is decreased continuously it gradually change its color and fluorescent blue without change in chemical composition. This size dependent optical properties is due to quantum confinement in which smaller particles will have larger band gap. [14]

![Fig: 2.1 Size dependent color fluorescent illustration of CdSe crystals of different sizes. As crystals get smaller, the fluorescent wavelength shortened.](image)

2.3 BLINKING OF SINGLE NANOPARTICLE

When the fluorescence emitted by a single nanocrystal was observed illuminating by light source, it was found that undergoes fluorescence intermittency or blinking characterized by the irregularly fluorescence switching between dark (OFF) and bright (ON) state. This phenomenon was reported by Nirmal and et al [15] for the first time on CdSe nanocrystal. Since then plenty of similar research have been stated in many fluorophores, single molecule, semiconductor nanocrystals of different shapes like sphere, rods and wires. [16–18]. Even the blinking
phenomenon is well accepted in wide range of particles, the mechanism of blinking is still not fully understood and there are different models which tried to address and explain the blinking processes. Among those quantum jumps in three levels system, charged trapped methods and time varying trapping rate with respect to fluorescence rate is described here.

2.3.1 Quantum jumps in three level systems

It became possible to observe the quantum jumps for first time in mid-1980s when trapping and optical spectroscopy of individual single ions were possible. When ions are illuminated by the light that resonant with both strong and weak transition. The former is dominant whose de-excitation has continuous fluorescence photons (bright/ON state) but the latter is less probable having longer period of de-excitation and interrupt the continuous fluorescence emission (dark/OFF state) as shown by fig 2.2a. Similarly, when molecules are illuminated it undergoes excitation and de-excitation cycles between singlet states resulting continuous fluorescence the bright state. The transition between singlet and triplet state is forbidden by symmetry but in practice they arise with small probability due to spin-orbit coupling. The decay from triplet state to singlet ground state as in fig 2.2b has longer period about microsecond to millisecond which results in interrupted fluorescence emission. Thus, quantum jumps in three levels provides the evidence of blinking. [19]

Classical excitation and deexcitation mechanisms are shown in the fig 2.2ii where a molecule can be excited by two-photons (2p) with any energy greater than the band gap between ground state ($S_0$) and first excited state ($S_1$). The molecules can return to the ground state without interacting with environment by emitting a photon or sometimes emitting heat by internal conversion. Natural fluorescence lifetime decay $t_n$ in the absence of radiation less decay is longer than the effective
lifetime decay $t_0$ due dissipation of energy in internal conversion. Over a large numbers of similar molecules measured the fluorescence decay is observed to be a single exponential. [20]

![Quantum jumps in three level system illustrating blinking behavior](image)

Fig: 2.2.i Quantum jumps in three level system illustrating blinking behavior[20]

![Absorption and emission from the excited state](image)

Fig: 2.2.ii Absorption and emission from the excited state [20]

### 2.3.2 Charge trapping model of blinking

This model described that blinking is result of charge trapping. Charge trapping refers to the phenomenon of localizing electron or hole at lattice defects at the core, interface or shell of Nanocystal. According to this model, when the nanocrystal is irradiated by the laser light it creates
an exciton followed by radiative recombination (as shown in fig 2.3. a) and remain in the ON state.

ON to OFF switching occurs when the nanocrystal charge is trapped due to either thermal ionization or photoionization (as in fig 2.3.b). The charged nanocrystal remains in OFF state despite successive absorption due to fast non-radiative (Auger) recombination where the energy transfer from the new exciton to lone charge take place (as in fig 2.3. c). OFF to ON switching take place when the trapped charge return to the core (as in fig 2.3.d). [21]

Fig: 2.3 Schematic representation of blinking by charge trapped model. CB=conduction band, VB=valence band and TS= trapped state. [21]

**2.3.3. Time dependent trapping rate the cause blinking**

In this model trapping rate\(k_t(t)\) is proposed to be the function of time dependent energy difference between the first and second conduction band. When the trapping rate \(k_t(t)\) is smaller than the fluorescence/radiation rate \(k_r\), it is ON state in which the photon absorption to create
exciton and radiative combination take place (as fig 2.4.a). when the trapping rate is faster than fluorescence rate, switching from ON to OFF state take place (fig 2.4.b). During OFF state the nanocrystals cycles between photon absorption creating exciton, removal of hole/electron to the trap and fast nonradiative recombination of the electron and trapped hole take place (as in fig 2.4.c). OFF to ON switching take place when trapping rate become slower again (as fid 2.4.d). [21]

![Diagram of blinking by trapping rate model](image)

**Fig: 2.4 Schematic representation of blinking by trapping rate model. CB=conduction band, VB=valence band and TS= trapped state. [21]**

### 2.4 SILVER COATED COBALT FERRITE

In this experiment we experimented Silver coated Cobalt ferrite (CoFe$_2$O$_4$) magnetic core shell nanoparticles as our sample. The size of single particle varies between 200-300 nm which was determined by a scanning electron microscopy (SEM) SU3500 system (Hitachi, Japan) image as shown in fig 2.5. They are ferromagnetic by nature and some properties are mention in section
2.1 and 2.2. They have high magnetic anisotropy [22] and these particles are highly interest in various biomedical imaging due to size dependent optical properties and hyperthermia and material designing applications (possibly LEDs and QDDs) as mentioned in introduction of chapter 2. Many organic fluorescent dyes and proteins are being used for the purpose of biological imaging [23] but they suffer photobleaching [24], due to which inorganic fluorescent with less photobleaching and high resistance to chemicals are ideal candidates for this purpose. Cobalt ferrite is the promising and ideal candidate for such phenomenon due to its properties.

Fig: 2.5 SEM image of the nanoparticle showing their size at different magnification.

2.5 EXPERIMENTAL METHODS

2.5.1 Sample preparation

Silver coated Cobalt ferrite magnetic nanoparticles and Ethyl Alcohol was taken in microcentrifuge tube (1.5mL MCT Graduated Natural) and sonicated in vortex mixer (fisher Scientific) for 5 mins. The particles dispersed and were in the form of suspension. These particles drop casted over quartz silicate glass slide using lambda-plus pipettor. The Alcohol dried up
instantly leaving the particles dispersed in substrate. Glass Cover slips was used to cover them and tapped using Scotch tape from all side of cover slip to prevent water leakage into the sample as the lens used in two-photon is water immersive. Proper care was taken while preparing the sample so that the particles are well dispersed. For that purpose, white light image of such nanoparticles was taken to confirmed that the particles were well dispersed as shown in figure 2.6. Fig a) shows agglomerated particles and by increasing the volume of alcohol, we can obtain fig b) which shows well dispersed particles.

Fig:2.6 White light image of a) agglomerated sample and b) well dispersed sample on glass slide

The samples are mounted on a three-dimensional motorized stage (Shutter Instrument, model MP-285) which allows us to record images and videos at a given z-position. When the well dispersed nanoparticles were observed under two-photon set up as shown in fig 1.4, it is found that there was fluctuation of the intensity of each nanocrystal over period. One pattern of intermittency is shown in the images of fig 2.7 taken in the interval of 15 sec. we split the channel using Image-J and green channel only is shown in the fig 2.7.
Fig: 2.7 Two-photon image depiction of the switching among fluorescent (ON) and nonfluorescent (OFF) state periods of nanocrystals with time under excitation.

2.5.2 Data obtaining

We used femtosecond (Maitai Ti-sapphire, Spectra-Physics) laser light source with pulse rate of 100fs. The laser power used was 70mW and objective lens of (Olympus) of 60x and NA 1 was used to irradiate the sample. The fluorescence was collected for different length of time from 1min up to 5min long movies through the same lens. After filtering in the dichroic mirror, the emitted fluorescence reached the PMTs. The red and blue PMTs channel were set off and recorded through green channel only in order to reduce the file size of our data. We used Image J (1.5 Oi) to analyze the recorded video where a single nanocrystal emitter intensity at each frame was determined by plotting Z- axis profile. After obtaining the data points the background signal (noise) shown by blue line in fig 2.8 was obtained from the nearby bare region of the substrate. The average of background signals was subtracted from each data points to confirm the signals is only from the nanocrystal blinking.
2.5.3 Bin time and threshold

The mechanism of switching processes causing the nanocrystal intensity to alter between ON and OFF states were probed using the distribution of these states in time scale. The blinking traces of single nanocrystal was generated using bin time of 30ms figure2.8. The intensity traces of more than 50 nanocrystals were analyzed. As particle undergoes fluctuation in the intensity, an intensity threshold ($I_{th}$) was defined such that the intensity below which it is said to be OFF state and above which it is ON state. The value of threshold varies widely on the process how it is defined. There are various methods described to find the intensity threshold among which one of them is to find the mean ($\bar{x}$) and standard deviation($\sigma$) of background signal of substrate. Then the threshold is set few times of standard deviation above the mean. i.e. ($I_{th}$) = ($\bar{x}$) + n $\sigma$ where n is number generally between 3 to 7. Another way is to define a threshold mid-way Center/Physical
threshold from the histogram plot of intensity vs frequency plot (fig 2.9) if the peaks were well separated showing OFF state and ON state distributions.

![Histogram plot of intensity vs frequency](image)

Fig: 2.9 Intensity vs frequency distribution of the given set of data, the red line shows the threshold and black line the background

This method can be employed easily if there are well separated peaks for the ON and OFF states. However, if there are not well separated two peaks for bright and dark states in the peaks of histograms, central threshold cannot be defined precisely. When the frequency is randomly distributed lacking well separated OFF and ON states in histogram-intensity plot. Poisson threshold had also been proposed by Catherine et al [25] to obtain the threshold value. By setting threshold the data set was divided into sequence of OFF and ON probability distribution given by

\[ P(t_{off(on)}) = \frac{N(t_{off(on)})}{N_{tot}} \frac{1}{\Delta t} \]  

(2.1)

This allows us to find the percentage time spent in ON/OFF state by that nanocrystal.
Where \( N(t_{\text{off(on)}}) \) is the number of OFF(ON) events of duration at \( t_{\text{off(on)}} \), \( N_{\text{off(on)}}^{\text{tot}} \) is total number of OFF (ON) states observed in the given time and \( \Delta t \) is the average of the time intervals to the preceding and following events. For rarely occurring events the probability distribution is defined by using weighted scheme to the probability density in which \( \Delta t \) in equation (2.1) is replaced by

\[
\Delta t = \frac{a + b}{2}
\]

Where \( a \) and \( b \) are the observed shortest and longest duration in the time-intensity graph. This approximation is very good when the events density is very low in a given bin time but for enough events in given time, we can simply use \( \Delta t \). For example, in our case we use bin time of 30ms and for equally spaced events both \( a \) and \( b \) are 30ms so \( \Delta t \) is 30ms as well. But for rare events both \( a \) and \( b \) must be greater than 30ms, therefore \( \Delta t \) is greater than 30ms which gives the true weighting probability distribution for OFF and ON statistics.

2.6 RESULTS

2.6.1 Off state probability statistics

Sample blinking data shown in fig 2.8 in fluorescence time series for a single nanocrystal showed distinct ON and OFF states. To quantify this emission intermittency OFF time and ON time probability densities \( P(T_{\text{off}}) \) and \( P(T_{\text{on}}) \) were generated using equation (2.1) before which an intensity threshold was determined as mentioned in section 2.5.3. The plot in fig 2.9 shows high frequency of events with OFF time \( (T_{\text{off}}) \) near the minimum integration bin width which become sparse and wider later in ON time \( (T_{\text{on}}) \). The semi log plot of \( P(T_{\text{off}}) \) with \( T_{\text{off}} \) when plotted as fig 2.10 a) is strong curvature. This graph shows distributed about \( 10^3 \) in probability and few
seconds on time. If the blinking behavior is governed by a single constant rate, it was expected a straight line (mono-exponential decay) on this plot but it was found to be multi-exponential.

But the log-log plot of the OFF-probability distribution showed a straight line over more than 2 decades of time and more than 3 decades of probability density as shown in figure 2.10 b). This pattern was repeated for all the nanocrystals that had been investigated. This plot fits to simple inverse power law given by

\[ P(T_{off}) \propto \frac{1}{T_{off}^m} \] (2.2)

Where m is the slope of the curve which varied from particle to particle between 1 to 2.3 in our investigated nanoparticles. It is worthwhile to note that such power law kinetics is inconsistent with any single exponential rate process as explained by section 2.31 of this thesis. Due to lack of structure in log-log plot, this data cannot assert on dynamics like slower/ faster but the blinking recovery rate in this nanocrystal must distributed over more than \(10^2\) fold of OFF time.
probabilities. The power law distribution is indicative of highly distributed OFF to ON switching rates.

2.6.2 On state probability statistics

The ON time probabilities density was also analyzed using the same procedure done for the OFF time. It was found widely distributed nature in semi log plot of ON time ($T_{on}$) vs ln ($P_{on}$) fig 2.11a) which spanned from more than 4 decades in probability and about 8 s in time. But the double log plot varied drastically as compared to OFF state distribution in which inverse power was seen for certain initial short period of time, but for longer time period it truncated to exponential form fig 2.11b).

![Fig 2.11 a) Semi log plot of ON time probability distribution](image1)

![Fig 2.11 b) log-log plot of ON state probability distribution and the red line is fitted to truncated power law](image2)

Fig: 2.11 a) Semi log plot of ON time probability distribution b) log-log plot of ON state probability distribution and the red line is fitted to truncated power law.

ON time probability distribution fitted to truncate power law given by

$$P(T_{on}) \propto \frac{1}{T_{on}} e^{-\frac{T_{on}}{\tau_c}}$$  \hspace{1cm} (2.3)
The time at which the power law starts to change into exponential is called the truncation time ($t_c$) which was found varying from particle to particle in our experiment, $m$ is the slope. The switching of power law to exponential law for long duration in ON state probability distribution could be the indication of either switching from ON to OFF take place with constant rate or saturation in the distributed rate that cause power law for short time.

Among the examined nanoparticles they behaved differently showing own blinking behavior in which the exponents for both OFF state and ON state changed and the truncation time for the ON state also changed. Among the examined nanoparticles randomly selected ten nanoparticles with their exponents and truncation time is listed in the table 1.2. From the table it can be inferred that exponents lie between 1 and 2 in most of the cases and sometimes it went below 1 for ON state and went above 2 for OFF state.

Table 1.2: Slopes of OFF states and ON states with truncation time for the ON state.

<table>
<thead>
<tr>
<th>Slope of OFF state ($m_{off}$)</th>
<th>Slope of ON state ($m_{on}$)</th>
<th>Truncation time of ON state ($t_c$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.76</td>
<td>1.16</td>
<td>1.43 s</td>
</tr>
<tr>
<td>1.14</td>
<td>1.07</td>
<td>1.32 s</td>
</tr>
<tr>
<td>1.52</td>
<td>1.11</td>
<td>1.26 s</td>
</tr>
<tr>
<td>1.69</td>
<td>1.13</td>
<td>2.54 s</td>
</tr>
<tr>
<td>1.63</td>
<td>1.25</td>
<td>1.54 s</td>
</tr>
<tr>
<td>2.3</td>
<td>0.92</td>
<td>1.94 s</td>
</tr>
<tr>
<td>1.39</td>
<td>1.37</td>
<td>1.41 s</td>
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<td>1.37</td>
<td>0.98</td>
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</tr>
<tr>
<td>1.56</td>
<td>1.05</td>
<td>1.17 s</td>
</tr>
<tr>
<td>1.7</td>
<td>1.17</td>
<td>2.29 s</td>
</tr>
</tbody>
</table>
Chapter 3: Discussion

The simple model that describes blinking behavior of single nanocrystal molecule is the quantum jump model developed by Neil Bohr [20] in 1913. In this model cycling of fluorescence between ground state $|1\rangle$ and excited state $|2\rangle$ take place followed by infrequent jumps to non-fluorescent triplet state $|3\rangle$ which ultimately recovers to the ground state. In this three-level system as shown in figure 2.2 where singlet – triplet inter system crossing leads to blinking due to larger time of triplet state. According to quantum jump model localization of nanocrystal and its switching between OFF and ON states with a single rate $\gamma$. The survival probability of such initial OFF or ON state can be written as

$$N_{on/off}(T) = e^{-\gamma_{on/off}t} \quad (3.1)$$

But experimentally we measured the probability density from $t=0$ to $t_{on/off}$ whose integral from $t=0$ to $t_{on/off}$ provides the survival probability. We can take the derivative of equation (3.1) to obtain the probability density, i.e

$$P(t_{on/off}) = \gamma_{on/off} (e^{-\gamma_{on/off}t}) \quad (3.2)$$

For simple kinetic model of constant rate, $\gamma_{on/off}$ it is expected to have single exponential behavior or in other words when semi-log plot of $T_{on/off}$ with $P(T_{on/off})$ should be linear. But from our experimental observations (fig 10.a and 11.a), it didn’t show such nature. It instead follows simple inverse power law for $P(t_{off})$ and truncated power law for $P(t_{on})$(as shown in fig 2.10.b and 2.11.b). This observed power law indicates that there must be broad distributions of rates for both the switching from OFF to ON and ON to OFF instead of single rate. Moreover, the observed range about $10^3$-fold on time scale from milliseconds up to even seconds would be a strong
evidence against three level quantum jumps which has faster relaxation life time from nanosecond for fluorescence and up to microsecond for phosphorescence [20,26].

The study of nanocrystal blinking on the probability distributions for OFF and ON state exhibited inverse power law behavior over decades of order. This is inconsistency with the predicted single exponential due to photoionization and neutralization followed by radiative recombination. There should be broadly distributed kinetics happening within the nanocrystal which leads to power law behavior. To describe this power law Verberk et al [27] proposed a static distribution of trap state at varying distance from the center of nanocrystal core. An electron undergoes tunneling in such trap. According to it charged nanocrystal is OFF state and neutral nanocrystal is ON state.

![Fig: 3.1 a) Auger recombination b) distribution of static traps at varying distance from nanocrystal core](image)

The charged nanocrystal remains in OFF state due to Auger recombination. Thus, static distribution of the trap state within the core of nanocrystal at different distance gained a broad range to neutralize the core and explained the widely distributed OFF state probability density. However, a single mechanism of switching from ON to OFF state is predicted by this model which cannot address the obtained experimental results. In the Auger recombination the energy release during non-radiative recombination is transferred to the lone charge of the core (fig3.1. a) that may expel it to another distance within the core, in the shell of the nanocrystal or even in another energy
state. This leads to neutralize the core resulting ON state. Moreover, the charge that reached to the surface acts as an electrostatic barrier for additional charge trapping (coulomb blockade) which leads elongated ON state.

Another explanation proposed by Kuno et al [28] is successful in explaining both OFF and ON state blinking and also power law over decades of magnitude on the time and probability density. According to this model, an electron tunnels between nanocrystal core and traps but the height and width of the tunneling barrier vary with time. OFF state is due to Auger recombination of the charged exciton. The broad distribution tunneling rates was obtained by them with modulating height and width of the tunneling barrier. The change in height and width was proposed to be due to change in conformation of nanocrystal by ligands used to passivate the surface of nanocrystal separating from external traps. The barrier height would physically represent change in molecular conductivity between nanocrystal and the surface orientation. The barrier width may represent the spatial fluctuations in the availability of traps sites far from the nanocrystal.

![Figure 3.2 Variation in height and width of tunneling barrier with time](image)

Other models of dynamic trapping of the charge had been reported by various researchers to explain the power law. Among which energetic diffusion of the trap state [29], spatial diffusion
of the trapped charge [30] and diffusion of the trap state and nanocrystal energy levels [31] are few of them. For all these diffusion processes, the probability for ON and OFF state were proposed according to 1D random walker to return its original position, which naturally leads to power law with slope 1.5. This seems to be consistent with the obtained experimental results to some extent where slope varies between 1 and 2.

3.1 CONCLUSION

Cobalt ferrite nanocrystals are luminescent, resistance against chemicals and photobleaching as compared to other organic dyes [32]. These properties make the nanocrystal for multiple applications such as molecular detection, imaging, and diagnostics. Although nanocrystal possess better optical properties, blinking is observed on the fluorescent emitted by single nanoparticle. The blinking is undesired event as nonfluorescent state is deprived of the signals and loss of information. Similar blinking phenomenon was observed in the Cobalt ferrite nanocrystal when excited by two-photon. The phenomenon of blinking in core-shell nanocrystal is explained in sub-section of 2.3. By setting an intensity threshold the observed fluorescence by a single nanocrystal was divided into OFF and ON states. The probability distribution of each of these states was analyzed which help to find the percentage of time spent on that state. The results show that blinking in nanocrystal cannot be explained by three level quantum jump model for blinking. By analyzing the probability distributions of OFF state fitted to inverse power law $P(T_{off}) \propto \frac{1}{T_{off}^m}$ (fig 10.b) indicates broad range of switching process from OFF to ON state.
ON state also inverse power law distributed for short time and get exponential truncated for longer period of time fig 11.b. Thus, ON state fitted to truncated power law $P(T_{on}) \propto \frac{1}{T_{on}^{m}} e^{-\frac{T_{on}}{r_{c}}}$. The exponential truncation could be indication of single transition rates from ON to OFF state or saturation in the distributed rate causing inverse power law for short durations. The power law was explained as charge trapping and Auger recombination, distribution of traps states, diffusion of excited states can be found on section 3 Discussion of this thesis.

![Graph showing comparison of percentage spent on OFF and ON state for different nanocrystal](image)

Fig :3.3 Comparison of percentage spent on OFF and ON state for different nanocrystal

In this study of blinking it was found that Cobalt ferrite nanocrystal remain in OFF states up to few seconds which is less than ON state (fig 3.3.) Despite its less percentage on OFF state as compared to ON state it is still significant which can create difficulties to obtain the whole information on imaging. Our next work would be advancing nanocrystals that suppress blinking and taking advantage of their distinctive optical properties of such nanocrystals.
References


Vita

Rajen Kumar Goutam was born and raised in Nepal. He obtained an M.S. in Physics from Tribhuvan University, Nepal in 2014. Then he came at University of Texas at El Paso to pursue higher study at the USA, led him to pursue an M.S. in Physics at the University of Texas at El Paso, Texas, USA. During the master's program, Rajen got involved in research activities at the Bio-photonics Laboratory of the Physics Department. He worked in interdisciplinary projects mainly focused on the applications of a Two-Photon Laser Scanning Fluorescence Microscopy. He recently got accepted into the Physics Program for doctoral students at the Clemson University where he expects to continue performing research in the field of physics.